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RP7214, a small molecule inhibitor of dihydroorotate dehydrogenase (DHODH), potentiates activity of Gilteritinib and Cytarabine in preclinical models of AML

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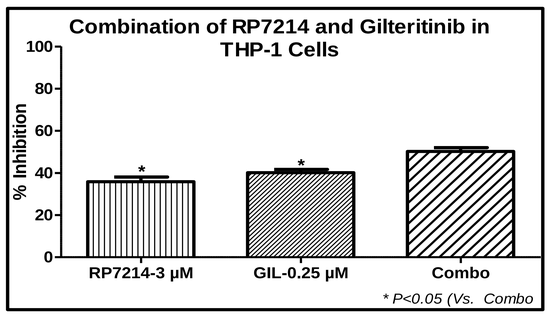
Introduction

Dihydroorotate dehydrogenase (DHODH), a rate limiting enzyme in pyrimidine synthesis, is overexpressed in certain cancers and impedes tumor cell proliferation *via* cell cycle arrest. RP7214 is a novel and potent DHODH inhibitor that inhibited DHODH activity in enzyme and PHA induced HWB/PBMC proliferation with IC_{50} and EC_{50} values of 7.8 & 2.5/0.60 nM respectively. The objective of this study was to evaluate the effect of combining RP7214 with Gilteritinib, a small molecule inhibitor of FLT-3, or cytarabine in preclinical models of AML.

	DHODH)	HWB	PBMC
IC_{50} (nM)	7.8	2.5	0.6
EC_{50} (nM)	-	2.5	

Table 1. DHODH enzyme activity was determined by its reduction of 2,6-dichloroindophenol during the oxidation of dihydroorotate using mitochondrial membrane preparations of U937 cells.

Freshly isolated human PBMCs or HWB were treated for desired concentrations of the inhibitor and induced with 2 µM PHA. CD4+ cells were determined after 48 h by flow cytometry



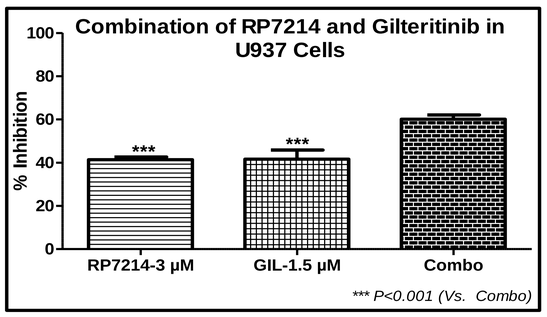
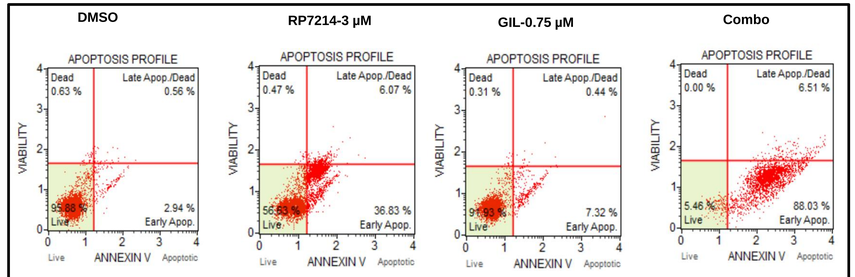


Figure 1. Combination of RP7214 and Gilteritinib in THP-1 and U937 cells. AML cell lines were plated in complete media at pre-determined density in 96-well plates and cells were simultaneously treated with RP7214 and Gilteritinib (GIL) for 72 hours. MTT assay was performed to determine the cell viability and Tukey's multiple comparison was performed using GraphPad prism (5.0) to determine the effect of combination. Addition of RP7214 potentiated Gilteritinib activity manifested by a significant (*P<0.05*) growth inhibition when compared to the activity of the individual agents



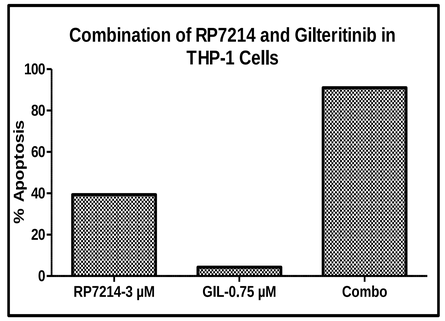
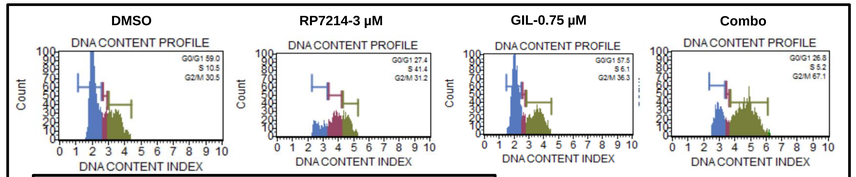


Figure 2. Apoptosis in THP-1 cell line. For measurement of apoptotic activity, cells were treated with desired concentrations of RP7214 and Gilteritinib (GIL) for 72 h and stained with Annexin and propidium iodide (PI) and analyzed on Muse cell Analyzer. ***Data demonstrated combination of 3 µM RP7214 + 0.75 µM GIL led to a 50% increase in the number of apoptotic cells***



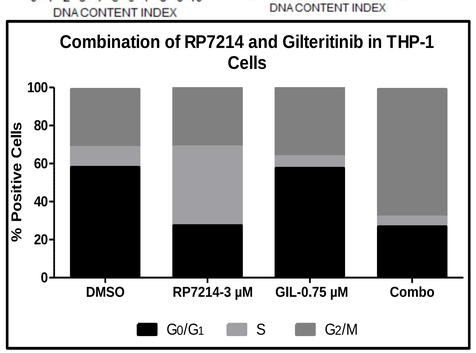


Figure 3. Cell Cycle Analysis in THP-1 cells. Cell lines were plated in media at a pre-determined cell density in 6-well plates Following overnight incubation, cells were treated with RP7214 and Gilteritinib. After 72 h, cells were stained with propidium iodide and analyzed by Muse cell analyzer ***RP7214-3 µM and Gilteritinib-0.75 µM caused 31% increase G2/M population.***

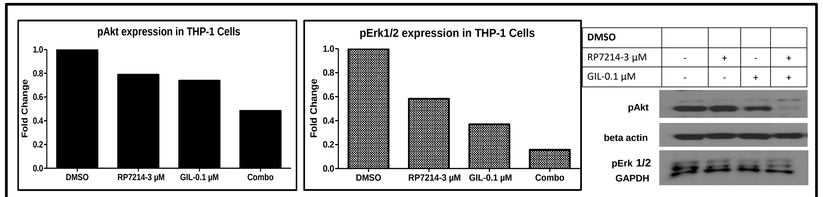
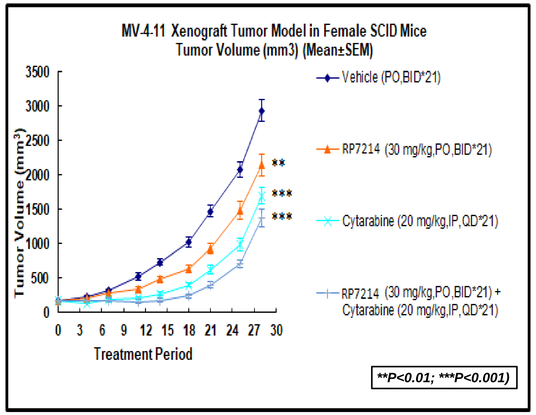


Figure 4. Expression of pAkt and pErk 1/2. THP-1 cells were plated in 1% FBS media at pre-determined density in 6-well plates and cells were incubated with RP7214 and Gilteritinib for 3 hours. Cells were lysed and western-blot analysis was performed to determine the expression of p-Akt and p-Erk-1/2. Combination of RP7214 and Gilteritinib-0.1 µM reduced pAKT and pERK by 51% and 58%, respectively, in THP-1 cells.



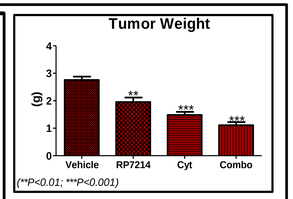


Figure 5.Anti-tumor activity in MV-4-11 Xenograft. RP7214 at 30 mg/kg/PO and Cytarabine (Cyt) at 20 mg/Kg, IP was tested in subcutaneous MV-4-11 human leukemia xenograft model. RP7214 in combination with Cytarabine demonstrated significant anti-tumor activities in both tumor size and tumor weight.

SUMMARY & CONCLUSIONS

- Inhibition of DHODH by RP7214 represents a unique therapeutic strategy in AML that accentuates the effect of approved and standard of care drugs such as Gilteritinib and Cytarabine
- RP7214 potentiated the activity of Gilteritinib in reducing the cell growth, induction of apoptosis, cell cycle arrest and inhibition of pAkt and pErk 1/2.
- RP7214 demonstrated anti-tumor activities manifested by a reduction in both tumor size and tumor weight in MV-4-11 human leukemia xenograft model when combined with Cytarabine
- The compound is currently being evaluated in IND-enabling tolerability studies with Phase-1 trial in AML expected to commence in 2020

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